REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. The changes to the claims are shown in attached Appendix A.

Cell membranes must allow passage of various polar molecules, including ions, sugars, amino acids, and nucleotides. Special membrane proteins are responsible for transferring such molecules across cell membranes. These proteins, referred to as membrane transport proteins, occur in many forms and in all types of biological membranes. Each protein is specific in that it transports a particular class of molecules (such as ions, sugars, or amino acids) and often only certain molecular species of the class. All membrane transport proteins that have been studied in detail have been found to be multipass transmembrane proteins. By forming a continuous protein pathway across the membrane, these proteins enable the specific molecules to cross the membrane without coming into direct contact with the hydrophobic interior of the lipid bilayer of the plasma membrane.

There are two major classes of membrane transport proteins: carrier proteins and channel proteins. Carrier proteins bind the specific molecule to be transported and undergo a series of conformational changes in order to transfer the bound molecule across the membrane. Channel proteins, on the other hand, need not bind the molecule. Instead, they form hydrophilic pores that extend across the lipid bilayer; when these pores are open, they allow specific molecules (usually inorganic ions of appropriate size and charge) to pass through them and thereby cross the membrane. Transport through channel proteins occurs at a much faster rate than transport mediated by carrier proteins.

Channel proteins which are concerned specifically with inorganic ion transport are referred to as ion channels, and include ion channels for sodium, potassium, calcium, and chloride ions. Ion channels which open in response to a change in the voltage across the membrane are referred to as voltage-sensitive ion channels.

Ion channels serve numerous physiological functions in excitable and nonexcitable cells. They transmit electrical signals to generate physiological cell responses. With electrophysiological recording techniques, a variety of ionic currents in many kinds of cells have been observed. The importance of these ionic currents has been demonstrated by pharmacological approaches using either naturally existing ion channel toxins or inorganic and organic ion channel blockers (such as local anesthetics). The essential physiological

roles of ion channels in normal cellular functions have been further strengthened by the link of diseases to defects in ion channel genes.

Over the past few years, molecular biological studies have revealed a large number of ion channel genes that could be responsible for the observed ionic currents. For example, there are more than 20 genes that have been cloned coding for voltage-gated potassium channels. Just within the Kv1 subfamily of the voltage-gated K^+ channels, there are at least seven members, and most of them (except Kv1.4) generate similar delayed-rectifier K^+ currents. Moreover, different potassium channel subunits can co-assemble to form heteromultimeric channels. Finally, the native complex of voltage-gated K^+ channels is also composed of accessory β-units and these β-subunits could convert the delayed-rectifier currents into rapidly inactivating A-type K^+ currents.

Antibodies have previously been used in functional studies of channels. Antipeptide antibodies, made against regions between S5 and S6 transmembrane segments of domains I and IV of the sodium channel α-scorpion toxin to sodium channels reconstituted in phospholipid vesicles or synaptosomes. It was not shown whether these antibodies could block sodium currents. An antipeptide antibody, by binding to a region in the intracellular loop between domains III and IV, slows sodium channel inactivation. Furthermore, it has been found that antisera from patients with Lambert-Eaton Myasthenic Syndrome (an autoimmune disease of neuromuscular transmission) could inhibit calcium channel currents. Antisera from some patients with Isaacs' Syndrome (acquired neuromyotonia) have antibodies against potassium channels and could increase neuronal excitability, possibly due to blocking of potassium currents. One monoclonal antibody that was generated against membrane fragments of the eel electroplax attenuates sodium current. Another monoclonal antibody that recognizes the dihydropyridine-binding complex in rabbit muscle transverse tubules inhibits calcium current in a mouse muscle cell line. However, in all these cases, the binding sites on the channel proteins was not clear.

The challenge now is to pin-point the underlying molecular identities (ion channel proteins) responsible for the observed ionic currents in native cells and to define their physiological functions. Although genetic manipulation with targeted deletion of ion channel genes would be helpful, the interpretation of results could be complicated by functional redundancy and developmental abnormalities. Some ion channel blockers are available, but they usually affect a group of ion channels and, thus, lack specificity towards one specific channel protein. These blockers were found empirically, either by clinical use or by broad

functional screening, rather than by rational design. The present invention is directed to overcoming these deficiencies.

The rejection of claims 1-9 and 19-27 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments and the following remarks.

To the extent that this rejection is based on the view that the phrase "external vestibule portion" is indefinite, applicant respectfully disagrees. Firstly, this phrase is defined on page 8 of the specification as being the portion of the ion channel located between the S5 transmembrane and pore forming region of the channel protein or between the pore forming region and the S6 transmembrane of the channel protein. This is clearly depicted in Figure 1A of the present application. The specific amino acid sequences for external vestibule portions in the Kv1.2, Kv3.1, and Kv3.1 potassium channels are set forth on pages 8-9 of the present application. To the extent the paragraph bridging pages 11 and 12 of the present application refers to variations of an "external vestibule portion", it does not render this phrase indefinite, because such variations are said to be within the external vestibule portion which is a small, well-defined region of an ion channel.

The literature's recognition of what is meant by the phrase "external vestibule portion" is extensive. For example, Aiyar, et. al., "Communication—The Signature Sequence of Voltage-Gated Potassium Channels Projects into the External Vestibule," J. Biol. Chem. 271(49): 31013-16 (1996) (attached hereto as Exhibit 1)("Aiyar") describes ion channels with external vestibule portions and modifications of such portions as follows:

> The ion conduction pathway, bounded at its external and internal entrances by wide vestibules, is formed in large part by the P-region and the C-terminal half of S6. The P-region of all cloned voltage-gated K^+ channels contains a highly conserved G(Y/F)GD motif, which is thought to constitute an essential part of the K^+ ion selectivity filter.
>
> The external vestibule is the receptor site for several scorpion peptide toxins that are potent blockers of K^+ current. In an earlier study, we used four structurally related scorpion toxins as molecular probes to map the topology of this region in Kv1.3, a voltage-gated K^+ channel that plays a vital role in modulating lymphocyte activation. This was achieved by determining the three-dimensional structures of the toxins and by identifying multiple pairs of interacting toxin and Kv1.3 residues. Knowing the disposition of these interacting toxin residues

> from their NMR structures, we were able to deduce the architecture of a 30-Å-wide and ~8-Å-deep vestibule at the outer entrance to the Kv1.3 pore.
>
> * * *
>
> In the present study, we have used this series of KTX-Lys^{27} mutants as a caliper to estimate the vertical distance between His^{404} in the external vestibule, and Tyr^{400} and Asp^{402}, two residues contained within the signature sequence of Kv1.3. Using site-specific mutagenesis coupled with the thermodynamic mutant cycle analysis, we assessed the interaction strength and estimated the distances between the terminal amines in each of these Lys^{27} analogs and each of the three channel residues. Our data suggest that Asp^{402} and Tyr^{400} lie in a shallow depression at the center of a wide saucer-shaped outer vestibule. Based on these experimental data, we have developed a modified molecular model of the outer mouth of the Kv1.3 pore. (citations omitted)

The term "probe" is defined on page 11, lines 8-13 of the present application as follows:

> The present invention also relates to probes found either in nature or prepared synthetically by recombinant DNA procedures or other biological procedures. Suitable probes are molecules which bind to the external vestibule portion of the ion channels identified by the antibodies of the present invention. Such probes can be, for example, proteins, peptides, lectins, or nucleic acid probes.

From this section of the present application, one of ordinary skill in the art would fully understand what forms a probe that "binds to the external vestibule portion of the ion channel and is effective to inhibit ion transport through the ion channel", as claimed, can take. Accordingly, the term "probe" fully satisfies Section 112 of Title 35.

One of ordinary skill in the art would fully understand how to determine whether the claimed antibody, binding portion thereof, probe, or ligand binds to the claimed external vestibule portion and blocks ion transport through the ion channel from the disclosure of the present application. Page 12, lines 20-23 references Huang, et. al., Cell 75: 1145-50 (1993) as teaching an assay for determining whether a particular material inhibits

ion transport through an ion channel. In addition, the Examples of the present application describe electrophysiological recording procedures and a dendrotoxin binding assay to identify materials that block ion channels to identify materials which block transport through ion channels and bind to outer vestibule portions (see pages 13-15 of the present application). Further, Examples 1-7, show actual studies of how materials block transport through ion channels and bind to outer vestibule portions for various ion channels. From this disclosure, one of ordinary skill in the art would be fully able to determine whether the claimed antibody, binding portion thereof, probe, or ligand binds to the claimed external vestibule portion and blocks ion transport through the ion channel.

The terms Kv ion channel, Kv1.2, Kv1.3, and Kv3.1 are fully described in the present application. Page 1, lines 8-30 of the present application describes channel proteins and identifies those which are specifically concerned with inorganic ion transport as ion channels (e.g., ion channels for potassium transport). Ion channels which open in response to a voltage change across a membrane are voltage-sensitive ion channels (page 1, lines 30-32 of the present application). Voltage-gated potassium channels (i.e. K_v channels) and the fact that many have been cloned are discussed on page 2, line 15-page 3, line 2 of the present application. The Kv1.2, Kv1.3, and Kv3.1 ion channels and sequence information for them are set forth in Figure 1, page 5, lines 9-30, and page 8, line 24 to page 9, line 7 of the present application. Therefore, contrary to what is said in the outstanding office action, the present application fully describes the terms Kv ion channel, Kv1.2, Kv1.3, and Kv3.1.

Further, the meaning of these terms is well known to those of ordinary skill in the art. For example, Pongs, "Molecular Biology of Voltage-Dependent Potassium Channels," Physiol. Rev. 72(4): S69-S88 (1992)("Pongs")(attached hereto as Exhibit 2) contains the following description at p. S69:

> The idea that outward potassium currents underlie the repolarization of action potentials orginates from the now classical electrophysiological studies of Hodgkin and Huxley. They proposed that depolarization of a membrane leads to a delayed activation of rectifying currents. The purpose of these currents is to repolarize the membrane. Hence, the channels through which these repolarizing outward currents flow were termed delayed rectifier channels. These channels are selective for K ions which permeate the pore of the channel ~30-fold better than Na or Ca ions.

> Therefore, these channels were termed delayed rectifier or voltage-gated potassium (K) channels. (citations omitted).

Specific potassium channels are described on page S76 (see particularly Table 4) and page S84 (see particularly Table 7).

Thus, the terms Kv ion channel, Kv1.2, Kv1.3, and Kv3.1 are fully understandable to one of ordinary skill in the art.

For all of these reasons, it is clear that the indefiniteness rejection under 35 U.S.C. § 112 (2nd para.) should be withdrawn.

The rejection of claims 1-8 and 19-26 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,827,655 to Chandy et. al., ("Chandy patent") is respectfully traversed.

The Chandy patent relates to the n K^+ channel expression product of the MK3 gene or functionally bioactive equivalent thereof and its uses, particularly in combination with identifying immune response and materials modulating or blocking them. To the extent that this reference describes identifying materials having a modulating effect on n K^+ channel expression, it involves providing an expression system containing the entire DNA encoding a functional K^+ channel expression product, contacting the expression system or the product of the expression system with one or more materials to determine their modulating effect, and selecting from those materials a candidate capable of modulating n K^+ channel expression (see col. 4, lines 3-14). Thus, the Chandy patent evaluates the effectiveness of an inhibitor against expression of the gene encoding the n K^+ channel expression product. Furthermore there is no indication which portion of expression product is monitored for binding by the inhibitor. By contrast, the present invention only needs to evaluate whether a particular material "binds to the external vestibule portion of the ion channel". No gene expression is called for by the claims, and, contrary to what is stated in the outstanding office action, there is no suggestion that the external vestibule portion is a site that should be monitored for binding of inhibitor candidates. As shown in Figure 1 of Pongs, the structure of an ion channel is intricate and has a number of different segments. There is absolutely nothing in the Chandy patent to indicate that binding at the external vestibule portion, as opposed to the S1, S2, S3, S4, S5, or S6 segments, should be monitored for binding activity. In view of these deficiencies, the rejection based on the Chandy patent should be withdrawn.

The rejection of claims 1-2 and 19-24 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 6,077,680 to Kem, et. al., ("Kem") is respectfully traversed.

Kem discloses methods and compositions comprising DNA segments and proteins from sea anemone species, particularly toxins, toxin analogs, chemically modified toxin analogs, and genes encoding toxins. In column 3, lines 9-25, the use of such toxins to interact with the P region of voltage gated potassium channels which is a short stretch of amino acids between the 5th and 6th transmembrane segments. By contrast, the claimed invention calls for evaluating whether a particular material "binds to the external vestibule portion of the ion channel". As shown in Figure 1 and described on page 5, lines 9-17 and page 8, lines 21-24 of the present application, the external vestibule portion and the pore forming region are distinct areas. With regard to Figure 1A, the pore forming region is shown within a box identified as the "P region", while the external vestibule portion is underlined. Figure 1B identifies the external vestibule portion as the amino acids which are shaded. This is entirely consistent with Figure 1A in that the amino acids underlined in the depiction of Kv1.2 are the same as those corresponding to the external vestibule portion in Figure 1B. The outstanding office action contains a great deal of discussion of what Kem teaches about ion channels and, particularly, Kv, Kv1.1, Kv1.3, and Kv3.1 channels. However, none of these teachings satisfy the requirement of the claimed invention that binding between the external vestibule portion and ion channel blocker candidates should be monitored. In view of this very basic deficiency of Kem, the rejection based on this reference should be withdrawn.

The rejection of claims 1-2 and 19-24 under 35 U.S.C. § 102(b) as anticipated by Stuhmer, et. al., "Molecular Basis of Functional Diversity of Voltage-Gated Potassium Channels in Mammalian Brain" EMBO J. 8(11): 3235-44 (1989)("Stuhmer") is respectfully traversed.

Stuhmer describes the cloning and sequencing of cDNAs isolated from a rat cortex cDNA library which encode several potassium channel forming proteins.

Nowhere does Stuhmer teach a method of identifying an ion channel blocker for an ion channel by "identifying, as an ion channel blocker for an ion channel, an antibody, binding portion of the antibody, probe, or ligand which antibody, binding portion of the antibody, probe, or ligand binds to the external vestibule portion of the ion channel and is effective to inhibit ion transport through the ion channel" (as set forth in claim 1) or a method of screening a drug for effectiveness as an ion channel blocker for an ion channel, wherein the ion channel has an external vestibule portion by "evaluating the cell to determine if the ion channel blocker candidate binds to the external vestibule portion of the ion channel and

inhibits ion transport through the ion channel" (as set forth in claim 19). Accordingly, the rejection based on Stuhmer should be withdrawn.

The rejection of claims 1-9 and 19-27 under 35 U.S.C. § 103 for obviousness over Kem in view of the Chandy patent, Chandy, et. al., "A Family of Three Mouse Potassium Channel Genes with Intronless Coding Regions," Science 247: 973-75 (1990)("Chandy article"), Stuhmer, Yatani, et. al., "A Monoclonal Antibody to the α Subunit of G_k Blocks Muscarinic Activation of Atrial K^+ Channels" Science 241: 828-31 (1988) ("Yatani"), Vassilev, et. al., "Identification of an Intracellular Peptide Segment Involved in Sodium Channel Inactivation," Science 241: 1658-61 (1988)("Vassilev"), and Tejedor, et. al., "Site of Covalent Attachment of α –Scorpion Toxin Derivatives in Domain I of the Sodium Channel α Subunit" Proc. Nat'l Acad. Sci. USA 85: 8742-46 (1988) ("Tejedor") is respectfully traversed.

The deficiencies of Kem, the Chandy patent, and Stuhmer are described above.

The Chandy article discloses a family of 3 mouse potassium channel genes and their sequences. Nowhere does the Chandy article teach a method of identifying an ion channel blocker for an ion channel by "identifying, as an ion channel blocker for an ion channel, an antibody, binding portion of the antibody, probe, or ligand which antibody, binding portion of the antibody, probe, or ligand binds to the external vestibule portion of the ion channel and is effective to inhibit ion transport through the ion channel" (as set forth in claim 1) or a method of screening a drug for effectiveness as an ion channel blocker for an ion channel, wherein the ion channel has an external vestibule portion by "evaluating the cell to determine if the ion channel blocker candidate binds to the external vestibule portion of the ion channel and inhibits ion transport through the ion channel" (as set forth in claim 19).

Yatani describes a monoclonal antibody that binds to a guanine nucleotide binding protein ("G protein"). However, a G protein is totally unrelated to a voltage-gated potassium channel. This distinction is reflected in Jan, et. al., "Annual Review Prize Lecture—Voltage-gated and Inwardly Rectifying Potassium channels," J. Physiol. 505.2: 267-82 (1997)("Jan")(attached hereto as Exhibit 3). In Figure 6 on page 272 of Jan, the structure of a voltage-gated potassium channel is depicted. Meanwhile, in Figure 8B on page 274 of Jan, the distinct structure of the G protein is shown. Moreover, Jan (page 273) teaches that "[i]t appears that the G protein βγ-subunits released by the activated M2 muscarinic acetylcholine receptor (m2 AChR) act directly on the muscarinic potassium

channel to cause channel activation". This phenomena and the distinct nature of the voltage-gated potassium channel and the G protein is shown in Figure 8 of Yatani. Thus, Yatani has nothing to do with the present invention's concept of examining the binding of materials to the external vestibule region of an ion channel.

Vassilev relates to the antibodies against a conserved intracellular segment of a sodium channel between transmembrane domains III and IV. This segment is in a completely different location than the outer vestibule portion. In particular, Vassilev's segment SP19 is intracellular (see Figure 1A on page 1658 of Vassilev), while the external vestibule portion, by virtue of its location between the S5 and S6 transmembrane regions has a more external location (see Figure 6 of Jan). Thus, Vassilev has nothing to do with the present invention's concept of examining the binding of materials to the external vestibule region of an ion channel.

Tejedor discloses the covalent attachment of α-scorpion toxin to an extracellular loop between transmembrane helices S5 and S6 of a homologous domain I of the sodium channel α subunit. However, as described *supra* with reference to Figure 1 of the present application, the area between the S5 and S6 segments contain not only the external vestibule region but also the P region. There is no suggestion in Tejedor that the binding of materials to the external vestibule region of an ion channel should be examined. Nowhere does the Tejedor teach a method of identifying an ion channel blocker for an ion channel by "identifying, as an ion channel blocker for an ion channel, an antibody, binding portion of the antibody, probe, or ligand which antibody, binding portion of the antibody, probe, or ligand binds to the external vestibule portion of the ion channel and is effective to inhibit ion transport through the ion channel" (as set forth in claim 1) or a method of screening a drug for effectiveness as an ion channel blocker for an ion channel, wherein the ion channel has an external vestibule portion, by "evaluating the cell to determine if the ion channel blocker candidate binds to the external vestibule portion of the ion channel and inhibits ion transport through the ion channel" (as set forth in claim 19). In fact, it was subsequently established that the scorpion toxin of Tejedor binds to the P region -- not the external vestibule portion. See Catterall, et al., "From Ionic Currents to Molecular Mechanisms: The Structure and Function of Voltage-Gated Sodium Channels," Neuron 26:13-25 (2000) (attached hereto as Exhibit 4), particularly page 14 which refers to various toxins as "pore blockers".

Since neither the Chandy article, Yatani, Vassilev, nor Tejedor overcome the above-noted deficiencies of Kem, the Chandy patent, and Stuhmer, this combination of references cannot form a proper basis for rejecting the pending claims.

On September 16, 1999, applicant submitted a Supplemental Information Disclosure Statement under 37 CFR §§ 1.97-1.98 with an accompanying PTO-1449 form listing the cited references. In the outstanding office action, the PTO-1449 was returned with the references crossed-off; no explanation for crossing off these references was given. Applicant maintains that the citation was proper and requests that these references be considered and that such consideration be reflected by initialing where they are cited on the attached copy of the PTO-1449 form. If the references were properly crossed-off, applicants respectfully request an explanation.

In view of all the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: September 13, 2001

Michael L. Goldman
Registration No. 30,727

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603-1051
Telephone: (716) 263-1304
Facsimile: (716) 263-1600

Certificate of Mailing - 37 CFR 1.8 (a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231. on the date below.

9/13/01 — Date

Wendy L. Harrold

APPENDIX

The changes made by the amendments to the present application are shown below with insertions being underlined and deletions being bracketed.

1. (Twice Amended) A method of identifying an ion channel blocker for an ion channel comprising:

providing an external vestibule portion of an ion channel [having an external vestibule portion] and

identifying, as an ion channel blocker for an ion channel, an antibody, binding portion of the antibody, probe, or ligand [specific to the external vestibule portion of the ion channel, wherein the] which antibody, binding portion of the antibody, probe, or ligand binds to the external vestibule portion of the ion channel and is effective to inhibit ion transport through the ion channel.

19. (Amended) A method of screening a drug for effectiveness as an ion channel blocker for an ion channel, wherein the ion channel has an external vestibule portion, said method comprising:

contacting a cell having an ion channel with a drug which is an ion channel blocker candidate; [and]

evaluating the cell to determine if the ion channel blocker candidate binds to the external vestibule portion of the ion channel and inhibits ion transport through the ion channel; and

identifying a drug which binds to the external vestibule portion of the ion channel and inhibits ion transport through the ion channel as an ion channel blocker.